

CARBAMATE PRODRUGS OF PHENYLETHYLAMINES: A NEUROCHEMICAL INVESTIGATION

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In our ongoing studies on the effects of N-alkyl substituents on the ability of CNS stimulants to enter and be cleared from the brain, we have prepared the ethoxycarbonyl (ethyl carbamate) analogues of 2-phenylethylamine (PEA) (an endogenous amine), amphetamine (AM), and tranylcypromine (TCP) (an MAO-inhibiting antidepressant).

An early study by Bjurulf et al. (1) on the N-ethoxycarbonyl analogue of chlorphentermine, a prodrug of the anorexiant chlorphentermine, showed that the prodrug had a "relatively prolonged effect which makes one dose in the morning apparently sufficient." The N-ethoxycarbonyl analogue of Normeperidine has been tested for its analgesic activity and found to show no toxicity and no physical dependence capacity (in monkey) (2). Several carbamate analogues (but not the N-ethoxycarbonyl analogue) of physiologically active amines, including AM, have been tested by Verbiscar & Abood (3). In their study they found the anorexiant property of the carbamates to occur with less central stimulation than with amphetamine and that the carbamates provided a sustained release effect. We have conducted preliminary neurochemical studies in brain on the ethoxycarbonyl derivatives of PEA, AM, and TCP, and the results of those studies are presented here. The structures of these derivatives are shown in Fig. 1.

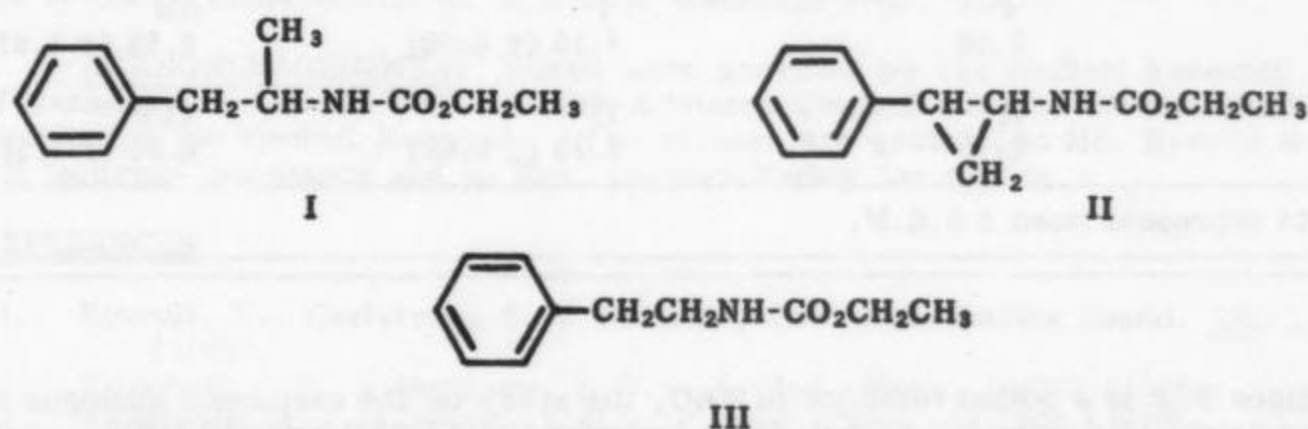


FIGURE 1: Structures of N-ethoxycarbonyl derivatives of amphetamine (I), tranylcypromine (II), and phenylethylamine (III).

METHODS: Male Sprague-Dawley rats (120-150 g) were injected intraperitoneally with the N-ethoxycarbonyl analogue of PEA (0.5 mmole/kg), AM (0.1 mmole/kg), or TCP (0.1 mmole/kg). The rats were sacrificed 1 h after administration of the appropriate compound, and the excised brains were frozen solid in isopentane on dry ice. Sample brains were stored at -40°C until time of analysis. Analysis was performed using gas chromatography with electron-capture detection (GC-ECD), with the amines and their N-ethoxycarbonyl analogues being converted to perfluoroacylated derivatives. Briefly, this consisted of extraction of the compounds from aqueous solution under slightly alkaline conditions and subsequent reaction with pentafluoropropionic anhydride.

The gas chromatograph was used with a ^{63}Ni electron capture detector. The capillary column used was 10 m OV-101, 0.8 mm o.d. and 0.25 mm i.d. Helium 2 ml/min was used as the carrier gas, and 10% methane in argon at flow rate 36 ml/min was used as make-up gas at the detector. The injection port and detector temperatures were both at 270°C. The column temperature was programmed from 80-270°C at program rate of 20°C/min.

For assay for MAO activity a modification of the procedure of Wurtman & Axelrod (4) was used, with ^{14}C -5-hydroxytryptamine as substrate for MAO-A and ^{14}C -phenylethylamine as substrate for MAO-B.

RESULTS & DISCUSSION: In the studies in which the rats were sacrificed 1 h after injection of the N-ethoxycarbonyl derivatives of AM and TCP, levels of 9.55 nmoles/g of AM and 8.95 nmoles/g of TCP were attained; these concentrations were somewhat higher than those found for the derivatives (4.15 nmoles/g for the AM analogue and 4.83 nmoles/g for the TCP analogue). These results are shown in Table 1).

TABLE 1: Concentrations of the N-ethoxycarbonyl analogues of AM and TCP and of AM and TCP in rat brain at 1 h after I.P. dose

	Dose of N-ethoxycarbonyl analogue (mmole/kg)	Concentration of N-ethoxycarbonyl analogue (nmole/g)	Concentration of active metabolite (nmole/g)
n = 6	I 0.10	I 4.15 ($\pm$ 0.58)	AM 9.55 ($\pm$ 1.63)
n = 6	II 0.10	II 4.83 ($\pm$ 0.68)	TCP 8.95 ($\pm$ 1.28)

Results represent mean $\pm$ S.E.M.

Since TCP is a potent inhibitor of MAO, the study on the carbamate analogue of this compound was extended to include an investigation of MAO activity. The results are listed in Table 2. In the brains of the rats treated with the TCP analogue as mentioned above, MAO-A and MAO-B were inhibited by 79.1% and 93.4% respectively. In other experiments conducted in parallel, concentrations of the TCP analogue and

TABLE 2: Inhibition of rat brain monoamine oxidase

Drug	In vitro		In vivo	
	Inhibition of MAO-A (%)	Inhibition of MAO-B (%)	Inhibition of MAO-A (%)	Inhibition of MAO-B (%)
II	11.7 $\pm$ 4.1	NIL	79.1 $\pm$ 3.4	93.4 $\pm$ 0.8
TCP	83.9 $\pm$ 5.5	99.0 $\pm$ 0.3	79.1 $\pm$ 7.0	90.8 $\pm$ 1.3

The results represent the means of 6 experiments. Concentrations of II and TCP *in vitro* were 4.8 μ M and 9.0 μ M, respectively, which were equivalent to the concentrations of these compounds found in brain in the *in vivo* study.

of TCP equivalent to the concentrations found *in vivo* (assuming that 1 g of brain is approximately equivalent to 1 ml) were tested *in vitro*. It was found that the ethoxycarbonyl derivative caused 11.7% inhibition of MAO-A and no inhibition of MAO-B, while TCP caused 83.9% and 99.0% inhibition of types A and B respectively. This suggests that the MAO-inhibiting effect observed *in vivo* is due not to the prodrug, but to the TCP formed from the prodrug.

In a preliminary *in vivo* investigation of the ethoxycarbonyl analogue of PEA, this drug was injected at a dose of 0.5 mmole/kg and the rats were sacrificed 1 h later. This larger dose was used so that the PEA formed could be measured; PEA, unlike AM or TCP, is rapidly metabolized by MAO. Under these conditions, brain levels of N-ethoxycarbonyl-PEA and PEA were 15.6 nmoles/g and 190 pmoles/g, respectively. In another series of rats in which PEA HCl (0.5 mmole/kg) was injected, brain levels of PEA were 835 pmoles/g. Since normal brain levels of PEA are 10-15 pmoles/g, the administration of the carbamate analogue may represent a method for selectively increasing brain concentrations of this amine without affecting levels of other biogenic amines.

In summary, it appears from this preliminary study that carbamate analogues may prove to be useful "prodrugs" of some bioactive amines, being able to alter the levels of these amines in, and their clearance from, brain.

ACKNOWLEDGEMENTS: Funds were provided by the Medical Research Council of Canada, the Alberta Mental Health Advisory Council and the Alberta Heritage Foundation for Medical Research. The authors are grateful to Ms. Brenda Murphy for technical assistance and to Mrs. Carolyn Farley for typing.

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